[Proc. Roy. Soc. Victoria, 43 (N.S.), Pt. II, 1931.]

Art. XIX.—*The Seasonal Sap Flow of Eucalyptus botryoides.*

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[Read 14th August, 1930; issued separately 28th February, 1931.]

Preliminary observations by A. O. Barrett (1927-8.)

After some years' observation in my garden at "Lalbert," Orrong Road, Armadale, Victoria, on the effect of a large number of dripping taps I gradually became aware that in those places where the "wet spots" caused by this dripping were adjacent to Eucalyptus trees, they tended to become dry areas in April to October, and then became wet spots again from November to March. On the other hand, those wet spots adjacent to European trees such as Poplars, Pines, Yews, Oaks, Oriental Planes, Chestnuts, etc., became dry in the late spring, summer, and early autumn, and remained wet during the remainder of the year.

I therefore decided to watch this action for twelve months during 1927-8, and found that my previous observations were correct. Details are, however, omitted for the sake of brevity.

The mean annual temperature is about 58° F., and the annual rainfall averages 25 5 inches.

The soil is a sandy loam on the surface, and beneath this is a fine sand, 98 per cent. of which will pass through a 50-mesh sieve, and beneath this again, at varying depths up to ten feet, is a clay mud.

Consideration of the above facts prompted the following investigation on three mature trees, specimens of *Eucalyptus botryoides*. The behaviour of these mature trees in my garden is entirely different from that of seedlings of from one year up to seven years old. During this period they form leaves, extend their branches, and increase the height of the main stem, and develop their root system throughout the year. If flower buds develop they are cast off while immature.

From eight to fourteen years they begin to pass into a new phase, and cease leaf formation in the winter (instantly if a crop of capsules form in March), but at about sixteen years of age they have become large trees, producing copious fruit buds and entirely ceasing to form new leaves or extending their branches from April until well into October. In all cases, however, sporadic short-lived developments may occur under abnormal conditions.

In August these mature trees develop tiny red beginnings of leaves, which do not enlarge until well into October. Thereafter they form successive flushes of leaves and extend their branches about every five weeks until the end of March. At Christmas they flower, and the white blossom is fertilized in January. By the end of March they are usually covered with capsules, and all leaf development ceases. In April an active growth of white roots begins which lasts until October in a normal season and then ceases.

Where, however, abnormally heavy rain falls in November, a sporadic development of fine roots will result. These roots are not more than 1/16th inch in diameter and seldom more than two to three inches long, and their appearance is unusual.

The hot weather then ensues with consequent surface dryness of the soil, and no digging will reveal any further white roots until the end of March.

Plan of Systematic Experiment.

In order to examine their habits in detail from the generalizations enumerated above, we selected three specimens of *Eucalyptus botryoides* which were growing normally and away from any dripping taps.

They had been grown from the same lot of seed, planted in 1913, and averaged thirty-five feet in height; their trunks were five feet in circumference, and the trees' branches had a spread of some twenty-five feet. They were healthy, full of vigour, and loaded with leaves. They were placed in a row about ten yards apart with other smaller trees and shrubs, but were not overshadowed by, or very close to, any other large trees.

They are indicated by the letters A, B, and C, and the only difference to be seen between them is that the outside dead bark of C is darker than that of the others, being at the North end of the row whilst A is at the Southern and more shaded end.

Experimental Methods.

Quantitative experiments on growing trees are difficult to carry out in such a manner that the experimental error does not vitiate the result, but it was decided to work with the three trees described, and to measure the variations of the water, ash (mineral matter), and sugar content of the bark from month to month throughout the year.

Sampling.

In the first place it was necessary to devise an instrument by which samples could be obtained each month, which would not only be comparable from tree to tree, but for each month of the year.

The taking of the sample must also interfere as little as possible with the growth of the tree. For this purpose a large "Cork-borer" was constructed of $\frac{5}{8}''$ mild steel, provided with a sharp saw-tooth cutting edge and fitted with an accurately machined plunger with which to push out the sample of bark when it had been cut.

To obtain a sample of bark one bores through the dead bark down to the living layers, then cleans the borer and bores gently through the living bark until the alburnum is reached, being careful not to express the sap water more than can be helped during the process. When the borer arrives at the alburnum it stops and slips round freely on its wet surface. By continuing the turning and pulling, the core obtained comes freely out and is gently pushed into the drying bottle, whose lid is immediately replaced. The boring is continued until from three to six borings of the living bark of each of the three trees have been secured.

In the summer there is no bleeding, but during the winter the bark contains so much sap that the pressure on the borer expresses some liquid even with the most careful manipulation.

The error due to this cause is insufficient to vitiate the conclusions we have arrived at as to the seasonal variation of the moisture content.

After much experimenting with other trees, the samples for the first month (August, 1928), consisting of at first three and afterwards six borings, were taken at a level of four to five feet from the ground, and about three to four inches apart from one another. Next month they were taken three to four inches diagonally lower so as not to be affected by the previous set.

As even a casual observation shows that there are some more or less dormant parts in the bark of any individual tree, and also parts that are more active, it was decided to take the samples in each tree from directly between the under side of a large branch and the top side of the root obviously supplying that branch. A similar position was chosen on the western side of each tree, and all samples were taken in that western quadrant in the direct line of the flowing sap.

Determination of moisture content of the living bark.

The weighing bottle containing each sample (5 to 8 grams at first, but increased to double that amount in later months) was placed in a drying oven at about 50°C. for about twelve hours; then the temperature was raised to 70°C. for two hours, and finally to 100°C. for two hours longer, or until weight was constant. This procedure was necessary to prevent the decomposition of any sugars present.

In this way the following figures were obtained for the samples taken on 8th September, 1928:—

	A.	B.	C.
Weight of sample	8·313	6·986	4 988
Loss of weight on drying	5 493	4·535	2.939
Percentage of moisture	66·0%	65·0%	59 9%

Samples were similarly taken and examined at the beginning of the second week in each month, and the moisture contents so obtained have been tabulated, with other data, in the Table and Graphs.

Graph I also shows the actual falls of rain as recorded at Caulfield and Prahran, the nearest meteorological stations to Armadale, during the period of the experiments. It will be seen that the rain has in some cases apparently shown its effect on the moisture content, and sometimes on the sugar and ash as well, but, generally speaking, the predominating influence has clearly been the seasonal habits of the trees only slightly modified by the local climatic conditions.

Determination of the ash and sugar contents.

The determination of the ash content of the dried bark was straightforward, and summaries of the results are recorded in the table and in Graph II.

The estimation of the sugar was a more difficult problem. The ordinary method of titration with Fehling solution may be seriously inaccurate when applied to materials containing other soluble matters than sugars, especially if only small and variable amounts of the sugar be present. For these reasons the suitability of some of the more recent methods has been investigated, and the experience gained related in some detail.

Trial of the Chloramine-T method.

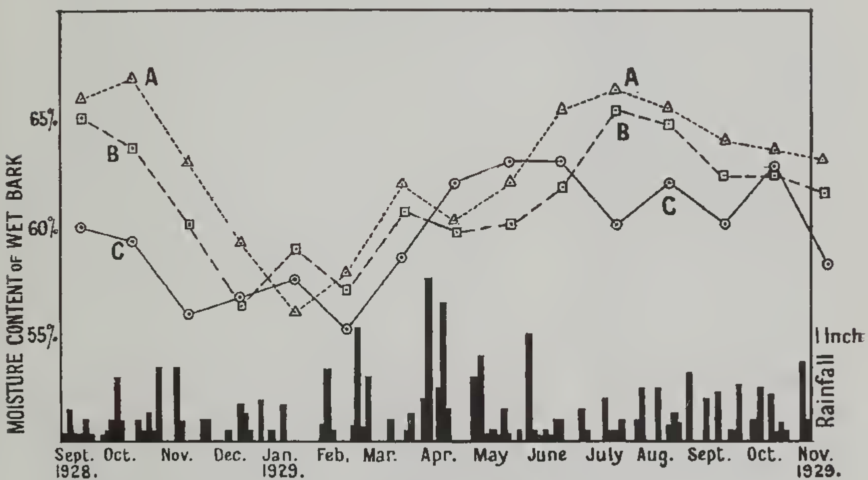
This method, in which a solution of chloramine-T in the presence of potassium iodide provides a mild oxidizing action, has several advantages, notably in its power of differentiating between the aldose and ketose groups of sugars.

Preliminary experiments soon emphasized the fact that accurate results can only be obtained when the proportions and balance of all the reagents are carefully adjusted, not only with regard to the concentration of the sugar to be determined, but also to the acidity of the solution and the amounts of iodide, chloramine-T, etc., used.

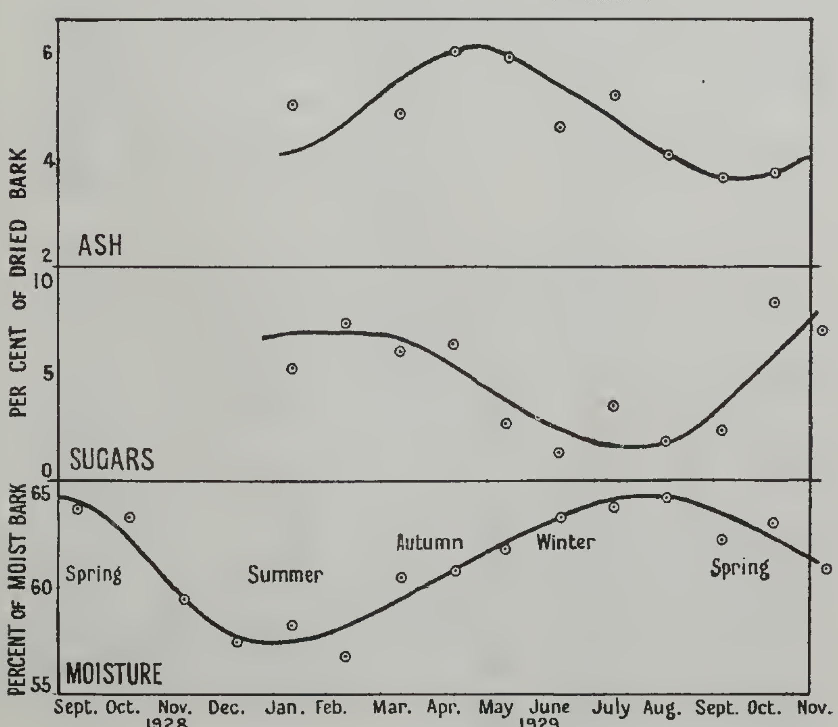
Variations in the temperature and duration, not only of the reaction itself, but also of the extraction of the sugars from the bark, were also found to be vital factors, and attempts were made to fix these conditions arbitrarily and carry out the estimation of sugar on the quantities of bark samples available.

After many tests, it was found that while quite accurate results could be obtained with pure sugar solutions by this method, the results for extracts of the bark varied so erratically with slight changes in the procedure that they could not be considered as giving any reliable measure of the sugar content. Although phospho-tungstate reagent was added in every case to remove tannins, albuminoids, etc., it is probable that other substances as well as sugar are present that are affected by the iodine oxidizing solution. The chloramine-T method was therefore discarded for our purpose, and attention was directed to the process of preparing the extract.

Graph I.—Moisture Content of Living Bark.



Graph II.—Average Values of the Ash, Sugar, and Moisture Contents of the bark of three trees.



SUMMARY OF THE ANALYTICAL DATA FOR THE THREE TREES.

Date of Sample	Tree	Moisture %	Sugar %	Ash %	Mean for 3 Trees Moisture	Sugar	Ash
1928.							
Aug. 9	A	65·25					
	B	65·07			63·6		
	C	60·5					
Sept. 8	A	66·1					
	B	56·0			63·7		
	C	59·9					
Oct. 10	A	67·0					
	B	63·8			63·4		
	C	59·4					
Nov. 10	A	63·0					
	B	60·0			59·6		
	C	55·8					
Dec. 10	A	59 3					
	B	56·4			57·4		
	C	56·6					
1929.							
Jan. 10	A	56·0	5·9	4·73			
	B	58·9	5·2	4·62	58·2	5·2	4·98
	C	57·6	4·45	5·60			
Feb. 9	A	57·8	6·7	3·50			
	B	57·0	6·55	3·15	56·6	6·95	3·50
	C	55·0	8·6	3·94			
March 14	A	62·2	3·8	4·07			
	B	60·5	6·2	4·55	60·4	6·0	4·85
	C	58·4	8·0	5·94			
April 10	A	60·2	4·75	6·06			
	B	60·0	7·45	4·29	60·7	6·3	6·02
	C	62·0	6·7	7·70			
May 11	A	61·8	3·7	5·46			
	B	60·0	2·05	5·48	61·6	2·5	5·95
	C	63·1	1·8	6·90			
June 11	A	65·3	1·1	4·58			
	B	61·6	1·8	—	63·3	1·25	4·58
	C	62·9	0·9	—			
July 9	A	66·3	5·45	5·96			
	B	64·8	3·2	5·79	63·9	4·4	5·19
	C	62·0	4·65	3·82			
Aug. 9	A	65·6	2·65	4·03			
	B	64·8	1·85	3·44	64·1	1·75	4·09
	C	62·0	0·75	4·80			
Sept. 17	A	64·0	3·1	—			
	B	62·8	2·6	—	62·3	2·4	—
	C	60·0	1·45	—			
Oct. 10	A	63·7	8·2	—			
	B	62·5	8·1	—	62·9	8·5	—
	C	62·6	9·25	—			
Nov. 12	A	63·0	6·95	—			
	B	61·4	7·2	—	60·8	7·05	—
	C	58·1	7·0	—			
Average for Period	A	62·9	4·85				
	B	60·95	4·85				
	C	59·6	5·8				

Preparation of bark extract.

It was soon found that the cores of bark were not in a form or condition that enabled them to be accurately sampled or completely extracted. However, after the experience gained in the chloramine-T tests the following procedure was developed and finally adopted for preparing the extract for analysis.

The dried material as received was in brittle lumps, but not very homogeneous; this was ground in a coffee mill and again dried at 100°-105°. Of this powder, 2·00 grams were weighed out into a flask, and allowed to soak in about 50 cc. of water over-night. Next day 5 cc. of phospho-tungstate reagent was added to precipitate reducing substances other than sugars, and the total volume made up to exactly 100 cc. This readily filtered, and the filtrate, or rather an aliquot of it, was used for the determination of the reducing sugars.

Trial of Fehling solution using methylene blue as indicator.

An attempt was made to use ordinary Fehling solution with methylene blue as the indicator of the end-point. This latter reagent, though requiring a little practice in its use, and necessitating the exclusion of air during the boiling process, is undoubtedly superior to the starch-iodide method of spotting the end-point.

The difficulty was soon met, however, that the amount of sugar available in the bark was generally much too small to reduce 5 cc. lots of Fehling solution, and that when lesser quantities of Fehling solution were tried the detection of the end-point became uncertain and the titration volumes were not proportional to the sugar content.

This method, therefore, also had to be discarded, and Clark's process was given a trial.

Trial of Clark's method.

Here the sugar is oxidized by boiling with an exact amount of Benedict's solution for exactly five minutes—the whole mixture occupying a definite volume (in our case, 40 cc.). The reduced cuprous oxide is determined by adding measured volumes of acetic acid, N/25 iodine, and hydrochloric acid, and titrating the residual iodine with N/25 thiosulphate, using starch paste as indicator.

This method, though apparently complicated, and requiring some ten measurements of volume, time, or weight for each determination, is capable of estimating much smaller quantities than the others. (The amount concerned in each titration actually ranged from one to eight milligrams.) Here, as in the chloramine-T method, a careful balance of the quantities is necessary, and the proportions of this balance had to be worked out for our present purpose.

Standardizing the solutions with pure invert sugar showed that even with this process the titration results are not quite proportional to the amount of sugar present, but a graph was drawn from which an accurate determination of the sugar content could be made in each case.

In conclusion, Clark's method was found to give consistent results with the bark extracts, and the estimations were done in duplicate, or oftener, if any doubt arose as to their substantial accuracy.

Unfortunately, the peculiar requirements of the estimation had not been recognized at first, and by the time the above procedure had been evolved, the material for the first three months had been used up: the results obtained with them were of uncertain value and have been discarded.

Each month an aliquot of the bark extract was also inverted with acid before titration, but the results obtained were merely duplicates of the untreated extracts, and confirmed the absence of invertible sugars.

The figures thus obtained by Clark's method for reducing sugars are recorded in the table and in Graph II.

Discussion of Results.

Tree A on the southern end of the row was somewhat shaded by the others, and the ground in which it was growing was also slightly lower, and possibly its roots had a sli htly damper feeding ground than either B or C. This difference shows itself definitely, though not invariably, in the moisture content of the bark, the average value of which over the period of experiment varies by some three per cent. from tree to tree.

No similar consistent difference shows itself in the sugar and ash contents.

The averages of the results obtained for all three trees have plotted in Graph II so as to summarize in one view as much of the data as possible.

Two results stand out clearly:—

1. In spite of any slight and temporary influence that the climatic factor may show, it will be seen that the moisture content of the bark reaches a minimum in February, and begins to rise in March until it attains a maximum in August, when it begins to fall again. It is then ready for the rise at the start of the next autumn.

2. The sugar contents (expressed as a percentage of the dry bark) fluctuate inversely as the moisture content. Were they expressed as a percentage of the wet bark, or of the water contents, this inverse relation would be still more marked.

It is also certain that these seasonal variations of the water content are not merely apparent variations due to corresponding alerations in the thickness (or volume) of the bark.

Although no attempt was made to measure the thickness of the bark from month to month, it obviously did not vary much from ⅝ inch in the part that was being sampled. It was also noted that in January the bark was so rope-like in texture that air-spaces were clearly apparent to the eye; in winter, on the other hand, these spaces were filled with water. In a wet year the living bark and alburnum are loaded with water by August, and the tree can hold no more from root-tip to leaf stalk.

As regards the concentration of sugar in the spring and summer, a simple calculation will show that this is a real accumulation, and not merely an arithmetical change due to variations in the percentage of water content.

Collateral Observations (1929-30.)

Simultaneously with the above experiments, the growth of the tree roots was also watched and investigated.

On February 1st, 1929, a large root from tree A was carefully uncovered from the trunk to its furthest extremity—about twenty yards away. As soon as possible it was all covered in again with the same soil, except that, in order to facilitate future inspection, the root-tips were first protected with cornsacks and then covered to their original depth with two inches of soil.

An examination of the root-tips showed the central extension of the main roots to be obviously alive, and covered with a thin red-brown bark having a sweetish taste. The old growing tips had died and were black and rotten. There was no sign of any active white roots or even of any growing root-tips.

On March 1st the earth under the sacking was quite wet and the sacking was rotting.

On April 1st new root apices were freely growing from the old root ends, but not from the apex itself in any case.

On May 1st the soil beneath was dry and the cornsacks were so pierced through with white roots that they could only be lifted by tearing the roots off.

On June 1st the sacking had so rotted that it had become part of the soil, so the spot was protected with a wire cage from disturbance till the next spring.

On September 1st the weather and the soil began to dry out.

On October 1st, 1929, no white roots could be found.

On February 1st, 1930, the entire system was uncovered as before, and this time protected with a galvanized-iron frame 8 feet long by 30 inches wide, and reaching 1 inch clear of the roots. Its roof was covered with sacking and soil, and it could be lifted in two sections and held up by a stake. The undisturbed soil was wet for three inches below the surface.

On March 1st the roots were still dormant, only two incipient root-tips being noticed.

On April 1st many sturdy white rootlets were visible.

On May 1st a multitude of white rootlets had appeared, and the ground was dry.

On June 1st white rootlets were still abundant and the soil quite dry, especially where the roots were most plentiful.

On July 1st another set of rootlets had begun to form, and it would appear that there are probably three different varieties of rootlets produced during the root-forming period.

On August 1st a few sturdy white roots remained, and these were covered with soft down-like hairs.

These types of root growth are being further studied.

On watching an individual rootlet, one finds that it has a short life—three weeks at the most. All through the autumn and winter these roots, perhaps ¼ inch in diameter, are steadily extending via the growing point, cuticularizing, and then often drying up and dying. At the same time the front, actively living portion, is sending out new roots with hairs, which drain the soil in that immediate location, and then they too shrivel up and decay.

When the living bark of the tree is full of the water sent up from these roots, *i.e.*, by the beginning of spring in a normal season, all the root extremities become dormant, and no white roots can be found during the spring or summer. They await the return of autumn.

Summary.

1. The roots of mature trees of *Eucalyptus botryoides* are apparently dormant during the spring and summer, but begin to form white, very active roots and root hairs in the early autumn.

2. During the autumn and winter, water is stored up in the bark, the moisture content of the living bark rising from 57 to 64 per cent. (*i.e.*, 132 to 178 per cent. of the dry bark), whilst the formation of new leaves is almost nil.

3. After the irrigative period the new leaves form in a succession of flushes or waves every three to five weeks throughout the spring and summer. At the same time, the sugar produced by these leaves accumulates in the bark until the sugar content has increased from 1·5 to 8 per cent.

4. The ash content is at its minimum (3·5 per cent.) in the spring, and at its maximum (6·0 per cent.) in the autumn.

5. It seems probable that these periods of root growth and the other peculiarities observed so different from those of the exotic plants of colder climates, are some of the developments which have enabled our Eucalypts to flourish in a semi-arid climate, withstanding a summer drought, and taking the opportunity of storing up a water supply in the wet season.

6. There are possibilities of the better use of Eucalypts, whether as firewood, as timber, or as grown to protect water-supply areas, that depend on an exact knowledge of the seasonal flow of sap in their roots and stems.

We hope this preliminary investigation will emphasize the need for systematic research on the subject.